We have examined the immunoglobulin gene configurations in cell lines from eight patients with diffuse histiocytic lymphoma in order to establish the cellular lineage and stage of differentiation of these lymphomas. The presence of heavy and light chain gene rearrangements as well as heavy chain class switching in seven cells placed these tumors within the B cell lineage. In contrast, one cell (SU-DHL-1), which lacks B cell-restricted surface antigens, retained germline heavy and light chain loci, indicating that it may represent a true histiocyte or uncommitted cell. Truncated RNAs for both the heavy and light chain immunoglobulins were responsible for the lack of surface immunoglobulin in the SU-DHL-2 cell line. Another cell line (SU-DHL-6), which possesses a t(14;18)(q32;q21) translocation, demonstrated an unexpected recombination within its heavy chain gene locus that may be the interchromosomal breakpoint.

THE DIFFUSE histiocytic lymphomas (DHL), as originally defined by the Rappaport classification system, represent a morphologically and immunologically heterogeneous subgroup of malignant lymphomas. They consist of a diffuse proliferation of relatively large mononuclear cells which tend to have vesicular nuclei and prominent nucleoli. Because of the marked heterogeneity of these lymphomas, their exact cellular origin was uncertain. Cell surface immunologic markers contributed enormously to clarifying this situation, revealing that many of the lymphomas were of clear B cell or T cell origin. Due to these insights, the NCI Working Formulation proposed to classify such cells as intermediate or high-grade diffuse cell lymphomas. Despite these advances, the exact cellular origin and stage of differentiation of many of these DHLs remained controversial. Many lack surface immunoglobulin (Ig) as well as definitive T-cell markers and have been called "null." It has been proposed that some of these may be "true histiocytes." Furthermore, several of these lymphomas which displayed mature B cell surface antigens failed to produce intact heavy (H) plus light (L) chain surface Ig and must possess molecular genetic defects accounting for this absence of Ig. Finally, several DHL cell lines have been noted to have chromosomal translocations at 14q32, also the site of the Ig H chain gene locus. Thus, such cells might display unexpected DNA rearrangements of their H chain gene locus and provide the opportunity to characterize a chromosomal breakpoint.

Because of these unresolved issues, we examined the Ig gene configurations and their expression within established cell lines from eight cases of DHL. The utility of Ig gene rearrangements as B cell-associated clonal markers is well-established. Although all mature B cells possess the mandatory rearrangement of H and L chain genes, other hematopoietic lineages tend to retain germline Ig genes. Specifically, the simultaneous presence of rearranged H plus L chain genes has thus far been an event restricted to B cells. Prior studies of human B cell precursors and mature B cell malignancies revealed a development sequence to Ig gene rearrangements in humans in which rearrangements of H chain genes preceded that of L chain genes, and \( \kappa \) rearranged before \( \lambda \). In this study, we show that most of these DHLs have rearranged H and L chain Ig genes and are committed to the B cell lineage. However, one cell had completely germline H and L chain genes and may represent a "true histiocyte" or an uncommitted cell. Two cases of B cell origin that lacked surface Ig production were further dissected by examination of their Ig RNA species. Finally, a cell line with a t(14;18)(q32;q21) chromosomal translocation was found to have an unexpected rearrangement within its H chain gene locus that identifies a chromosomal breakpoint.

MATERIALS AND METHODS

Determination of Ig gene configuration. We examined eight cell lines from separate patients with DHL that were previously established and characterized by Epstein et al, Winter et al, and Hecht et al. High mol wt DNA was extracted from each of these clonal cell lines. These genomic DNAs were digested to completion with the appropriate restriction endonucleases, size-fractionated over agarose gels by electrophoresis, and transferred to nitrocellulose paper. The genomic DNA blots were then hybridized to nick-translated or random-priming incorporated \(^{32P}\)DNA probes of the Ig gene fragments at specific activities of 200 to 800 cpm per picogram. After washing at the appropriate stringencies, the Ig gene patterns were visualized on autoradiograms. The human Ig genes were amplified and used as probes in Figs 1b and 2b. The initial configuration of the H chain genes was assessed with a 2.3-kilobase (kb) Sau3A germline gene fragment of the joining heavy (Jm) gene region hybridized to BamH1-digested or EcoR1-digested genomic DNAs. The status of the constant \( \kappa \) (\( C_k \)) and constant \( \gamma \) (\( C_\gamma \)) genes was assessed in BamH1-digested DNA with a 1.3-kb EcoR1 germline fragment of \( C_k \) or a 2.3-kb HindIII-Smal germline fragment of \( C_\gamma \), respectively (Fig 1b). The rearranged and germline forms of \( C_k \) genes were discriminated in BamH1-digested DNA probed with a 2.5-kb EcoR1 germline fragment of constant \( \kappa \) (\( C_k \)). The \( \lambda \) gene configuration was assessed in EcoR1-digested DNA hybridized with a 0.8-kb BglII/EcoR1 germline fragment of the constant \( \lambda \) (\( C_\lambda \)) gene capable of cross-hybridizing to all the \( C_\kappa \) genes (Fig 2b).

Analysis of Ig gene expression. Total cellular RNA was prepared from two of the cell lines (SU-DHL-2 and -9) by disrupting the cells with a Polytron (Brinkman Instruments, Westbury, NY) in the presence of 4 mol/L of guanidine thiocyanate. Total RNA was...
chain and both L chain gene classes (K genes). Development. In fact, all of the B cell-precursor leukemias restricted antigen B, yet lacked surface Ig. Figure 1 and examination of Ig gene configurations indicated that the Ig H chain and both L chain gene classes (K genes) were associated antigens (T3, T4, T6, T8, and T9). Commitment to B cell differentiation. Because T cell-associated antigens (T3, T4, T6, T8, and T9) are also missing, SU-DHL-1 may represent a "true histiocytic" lymphoma.

Three of the cell lines, SU-DHL-2, -8, and -9, displayed the B cell-restricted antigen B1 yet lacked surface Ig. Figure 1 and Table 1 reveal that these cells had rearrangements of their Ig H and L chain genes. The presence of H plus L chain rearrangements, HLA-DR, and B1, combined with the lack of common acute lymphoblastic leukemia antigen (CALLA), suggest that SU-DHL-2, -8, and -9 are at a mature B cell stage of development. Compatible with a mature B cell stage, all of these cells have undergone deletional H chain class switches. The Cg genes on both chromosomes have been deleted, and at least one of the JH regions in each of these lymphomas comigrates with a Ct region in BamHI-digested DNA (Table 1 and Fig 1). Thus, at least one allele in each cell line has correctly switched to a Ct region yet no γ chain protein is produced. The L-chain gene patterns were similar to those seen previously in other mature x-producing or y-producing B cells. SU-DHL-2 and -8, which have rearranged their λ genes, have no remaining germline k genes, whereas, SU-DHL-9 with a k rearrangement and some cytoplasmic k chain detected, has retained germline λ genes. These findings are consistent with the k before λ order to L chain rearrangements.

Despite the presence of Ig gene configurations characteristic of mature B cells in SU-DHL-2, -8, and -9, they all failed to assemble cell surface H plus L chains. Because adequate numbers of viable SU-DHL-2 and -9 cells were available, we were able to examine their RNA in order to determine if any defects in transcription would account for the lack of surface Ig production. Figure 3 indicates that SU-DHL-2 has abundant Ct RNA, but that it is abnormally small in size (1,100 base pairs) as compared to the normal-sized γ transcripts of

RESULTS

DHL-1 has germline H and L chain Ig genes. The SU-DHL-1 cell line displayed neither cytoplasmic or surface Ig, HLA-DR, nor the B cell-restricted antigen, B1 (Table 1). Its only B cell-associated marker was BA-1, and this antigen is not solely restricted to the B cell series. Examination of Ig gene configurations indicated that the Ig H chain and both L chain gene classes (k and λ) were retained in their germline form (Figs 1 and 2). H chain genes are known to rearrange very early within the pre-B cell stages of development. In fact, all of the B cell-precursor leukemias that have been examined to date have had H chain gene rearrangements. The earliest identifiable pre-B-cells have HLA-DR and rearrangements of only their H chain genes. The lack of HLA-DR and H-chain rearrangement in SU-DHL-1 indicates that it has made no discernible commitment to B cell differentiation. Because T cell-associated antigens (T3, T4, T6, T8, and T9) are also missing, SU-DHL-1 may represent a "true histiocytic" lymphoma.

Abnormal Ig RNA in DHLs with B cell surface markers, Ig gene rearrangements, but no surface Ig. Three of the cell lines, SU-DHL-2, -8, and -9, displayed the B cell-restricted antigen B1 yet lacked surface Ig. Figure 1 and

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1,900 base pairs (bp). No Cᵦ-containing or Cᵦ-containing messages were present (data not shown). In addition to the defective H chain RNA, SU-DHL-2 also had a truncated κ RNA of only 750 bp, compared to the normal size of 1,200 bp.

Many mature κ-producing B cells have been noted to generate small amounts of λ gene transcripts (S.J.K., unpublished observations, September 1983) even when their λ genes are in the germline configuration. Similarly, SU-DHL-2 displays detectable λ transcripts that are also smaller than those in neighboring counterpart cells in Fig 3. Thus, SU-DHL-2 has abnormally truncated messages for γ, κ, and λ, indicating the presence of multiple molecular defects and thus accounting for the lack of Ig production. In contrast, SU-DHL-9, which also lacks surface Ig and has undergone a H chain class switch, has an apparently normal-sized γ RNA and a normal-sized κ RNA (Fig 3). Some isolated κ L-chain is found in the cytoplasm of this cell line. Despite the presence of small amounts of normal-sized γ RNA, it fails, however, to produce γ chain, indicating that later defects, perhaps in translation, exist.

B Cell-type DHLs show Ig gene rearrangements compatible with a mature B cell stage. SU-DHL-4, -5, -6, and -7 all revealed rearranged H plus L chain genes (Figs 1 and 2, Table 1). Consistently, all of the cells shown to be κ producers had rearranged their κ genes, and the λ producer (SU-DHL-5) had the obligate λ gene rearrangement. However, an exception to the usual pattern of germline λ L chain genes in κ-producing cells was observed in SU-DHL-6 and -7. Both cells displayed λ rearrangements, despite being κ producers, which is a relatively rare event because κ genes usually rearrange prior to λ (Fig 2).

Both of the IgM-producing lines (SU-DHL-5 and -6) were noted to have one Cᵦ region which co-occupied the same BamH1 fragment with the rearranged Jᵦ. In the absence of a second Cᵦ region in these cells, the Cᵦ-containing alleles are by definition effective rearrangements of Vᵦ/Dᵦ/Jᵦ segments responsible for the production of IgM. The excluded H chain allele in both of these cells has undergone a H chain class switch in which a Cᵦ region co-occupies a BamH1 fragment with a Jᵦ segment. Thus, IgM-producing B cells can display H chain class switches on their opposite allele.
Table 1. Cell Surface Markers, Immunoglobulin, and Ig Gene Configurations

<table>
<thead>
<tr>
<th>Case No.</th>
<th>HLA-DR</th>
<th>B,</th>
<th>CALLA</th>
<th>Surface Antigens</th>
<th>Cytoplasmic Immunoglobulin</th>
<th>Cytoplasmic Immunoglobulin</th>
<th>Immunoglobulin Gene Pattern</th>
<th>Heavy Chain</th>
<th>x</th>
<th>λ</th>
</tr>
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<tbody>
<tr>
<td>SU-DHL-1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Germ</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SU-DHL-2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Germ</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SU-DHL-4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>IgG/k</td>
<td>IgG/k</td>
<td>1 Rearr</td>
<td>2 Rearr</td>
<td>Germ</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SU-DHL-5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>IgM/λ</td>
<td>IgM/λ</td>
<td>1 Rearr</td>
<td>2 Rearr</td>
<td>1 Rearr</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SU-DHL-6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>IgM/κ</td>
<td>IgM/κ</td>
<td>1 Rearr</td>
<td>2 Rearr</td>
<td>1 Rearr</td>
<td>2</td>
<td>1</td>
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<tr>
<td>SU-DHL-7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>IgG/κ</td>
<td>IgG/κ</td>
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<td>2 Rearr</td>
<td>1 Rearr</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SU-DHL-8</td>
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<td>+</td>
<td>-</td>
<td>IgM/λ</td>
<td>IgM/λ</td>
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<td>2 Rearr</td>
<td>1 Rearr</td>
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<td>1</td>
</tr>
<tr>
<td>SU-DHL-9</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>IgM/κ</td>
<td>IgM/κ</td>
<td>1 Rearr</td>
<td>2 Rearr</td>
<td>1 Rearr</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Germ, germline; Rearr, rearranged; Del, deleted; CALLA, common acute lymphoblastic leukemia antigen.

Surface antigens and immunoglobulin as presented in Winter et al.7

Furthermore, additional deletional rearrangements have occurred in the H chain gene region of SU-DHL-5. There are four functional human Cγ genes (γ1, γ2, γ3, γ4) and a nonexpressed pseudogene γγ.18,25 Some restriction fragment length polymorphism does exist so that the germline Cγ regions in humans occupy BamH1 restriction fragments of 25-kb or 13.5-kb, 12.5-kb, 11.8-kb, 10.0-kb, 9.4-kb or 9.0-kb, and 8.8-kb.26 Both gene copies of a number of these BamH1 fragments have been deleted in SU-DHL-5 and only a germline copy of the 12.5-kb fragment containing the Cγ1 gene has been retained (Fig 1). This indicates that Cγ deletional events have occurred on the expressed allele containing the Cγ region as well as on the excluded allele that has undergone a H chain class switch. The presence of a rearranged Cγ gene that lacks an associated JH region should provide an opportunity to identify the unexpected downstream rearrangement that is mediating this complex deletion (Fig 1).

The two cells that produce IgG (DHL-4 and -7) have undergone deleitional H chain class switches (Table 1, Fig 1). H chain class switches have occurred on both the expressed and excluded allele so that no Cγ regions are retained. Therefore, the IgG-producing DHLs are at a relatively mature stage of B cell development and have already undergone an H chain class switch. Thus, there is no evidence that these surface IgG-positive B cells have long, alternatively spliced transcripts which generate IgG, as has been proposed to exist for some B cells producing distally located isotypes.27

Chromosomal translocations in DHL may involve the Ig gene loci. Two of the cell lines examined (SU-DHL-4 and -6) were known to possess t(14;18)(q32;q21) chromosomal translocations. Previous extensive examinations of the t(8;14)(q24;q32) translocations of Burkitt's lymphoma revealed that the H chain gene locus at 14q32 was itself often mediating these translocation events. To determine whether the H chain gene locus was similarly involved in the rear-

Fig 3. Northern blot analysis of Cγ, Cδ, and Cε-containing transcripts in total RNA of SU-DHL-2 and SU-DHL-9. (C) A control B cell line producing normal-sized RNA as indicated by the dash marks. Truncated transcripts in SU-DHL-2 are noted by arrows. The higher mol wt bands represent precursor forms of the message present in the nuclear RNA portion.
rangements with 18q21, we examined the H chain genes in SU-DHL-4 and -6 to see if any unanticipated rearrangements had occurred. Specifically, we searched for rearrangements that could not be accounted for by the routine events of VH/DH/JH assembly or H chain class switch. Only the expected rearrangements of JH and C\_\alpha\_5 segments were observed in SU-DHL-4, typical of an IgG producer. A very unexpected rearrangement of VH/DH/JH assembly or H chain class switch. Only the events that could not be accounted for by the routine events had occurred. Specifically, we searched for rearrangements with 18q21, we examined the H chain genes in SU-DHL-6, however. This diploid cell line was known to have one normal chromosome 14 and a derivative 14q+ chromosome. However, Southern analysis revealed the presence of three instead of the usual two J\_\alpha-containing \textit{Bam} fragments (Fig 1). One of these J\_\alpha fragments (10-kb) comigrated with the C\_\alpha\_5 region in a \textit{Bam}H1 digestion, and thus corresponds to the effectively rearranged VH/DH/JH-C-containing allele responsible for the IgM produced and presumably is located on the normal chromosome 14 in this cell. We proposed that the other two J\_\alpha fragments (22-kb and 4.0-kb) were created by a chromosomal breakpoint within the J\_\alpha region of the phenotypically excluded allele. Consistent with this hypothesis, the 22-kb \textit{Bam}H1 fragment has an associated C\_\alpha\_5 region together with the J\_\alpha segment, whereas the 4.0-kb J\_\alpha-containing \textit{Bam}H1 fragment has no identifiable Ig gene information at its 3’ end (Fig 1). Subsequently, the 22-kb fragment has recently been shown to possess chromosome 18 information at its 5’ end and correspond to the derivative 14q+ chromosomal breakpoint.\textsuperscript{28} The 4.0-kb allele would thus possess chromosome 18 information at its 3’ end and would correspond to the derivative 18q− chromosomal breakpoint.


discussion

We have examined the status of Ig genes within DHLs. We have used their Ig genes as markers of cellular lineage, their state of differentiation, Ig production defects, and potential chromosomal translocation sites. The DNA rearrangements of Ig genes have proven of enormous value in assigning a B cell lineage to a number of lymphoid neoplasms, including the non-T form of lymphoblastic leukemia,\textsuperscript{12,16,23} hairy cell leukemia,\textsuperscript{13} and many lymphoma biopsy tissues.\textsuperscript{14,30} This is based on the fact that hematopoietic cells which pursue other than a B cell pathway of development usually retain their Ig genes in the germline form. Exceptions do occur for the H chain genes which occasionally rearrange in human T cells (2 of 23) and even myeloid cells.\textsuperscript{12,14} However, the L chain genes have been uniformly retained in a germline form in T cells, myeloid, promyelocytic, and monocytic cell lines and leukemias. Thus, the simultaneous detection of rearranged H plus L chain Ig genes serves as a strong marker for B cell lineage commitment. Since the histologic description of DHL, there has been considerable controversy concerning the cellular origin and state of maturation of the markedly heterogeneous affected cells. Many of the DHLs could be classified—by using immunologic surface markers, as T- or B-cell in nature—but others were placed within a “null” or true histiocyte category only by default.

In this study, we have examined the Ig gene configuration and expression in eight well-established and characterized cell lines. We show that most of these lines are at a mature B cell stage, possessing H plus L chain rearrangements, and that they may have already undergone a H chain class switch. Of the four that fail to make surface Ig, one (SU-DHL-1) retains germline H and L chain genes. Furthermore, two monoclonal antibodies (2H9, 1E9) produced by immunizing with SU-DHL-1 reacted with the nuclear membrane of histiocytes and interdigitating reticulum cells in normal lymphoid tissues.\textsuperscript{31} Moreover, SU-DHL-1 also displayed the monocytic marker Leu M5 and retained the same phenotypic markers after induction with phorbol ester.\textsuperscript{32} As noted above, SU-DHL-1 also lacks definitive B cell or T cell surface antigens. SU-DHL-1, therefore, provides an important cell line model that may be a “true histiocyte.” The other three cells lacking surface Ig (SU-DHL-2, -8, and -9) are clearly at a mature B cell stage of development and must possess molecular defects that prevent Ig production. Multiple molecular defects have been identified in SU-DHL-2, which has truncated H and L chain RNAs. This cell line may be an appropriate setting to search for a common molecular mechanism that results in the loss of Ig H and L chain production.

Several other observations concerning the configuration of Ig genes in lymphomas of mature B cell phenotype are noteworthy. Our examination of SU-DHL-4, -5, -6, and -7 indicate that the excluded, nonproductive H chain allele in these cells has undergone a H chain class switch. This is not unexpected in IgG producers in which the same molecular switching mechanism may be operative on both chromosomes. However, SU-DHL-5 and -6 are IgM producers, and their excluded allele has also undergone a H chain class switch to a C\_\alpha\_5 region. We have also noted this phenomenon at times in other mature IgM-producing B cell lines and leukemias. This raises important questions concerning the regulation of this event and its purpose for the cell. Furthermore, it provides a very clear marker to distinguish on a Southern blot which H chain allele is indeed responsible for Ig production. The L chain genes in humans appear to rearrange in an ordered sequence in which \kappa genes precede \lambda genes.\textsuperscript{12,14} A consequence of this is that \lambda genes are usually retained in their germline form within \kappa-producing B cells.\textsuperscript{15,32} SU-DHL-6 and -7 are \kappa producers which show clear exceptions to this general pattern, having \lambda gene rearrangements. Whether these were present prior to transformation or were acquired in vivo or in vitro is uncertain, but they do indicate that \lambda rearrangements can at times occur in \kappa-producing cells. We have recently identified a \kappa-deleting element (\textit{ede}) which is rearranged to disrupt the \kappa locus prior to the time of \lambda gene rearrangement.\textsuperscript{33} SU-DHL-6 has rearranged a copy of this \textit{ede} on its excluded \kappa gene allele. The rearrangement of the \textit{ede} may prove to be related to the progression from \kappa to \lambda rearrangements.

Two of the DHL cell lines (SU-DHL-4 and -6) possess chromosomal translocations t(14;18)(q32;q21) usually associated with the nodular (follicular) type of lymphomas.\textsuperscript{9,34} However, these diseases may prove to be a continuum because some lymphomas that initially present with nodular histology progress to a diffuse architecture over time.\textsuperscript{35} The chromosomal break at 14q32 is the same band in which the
human H-chain genes are located.\textsuperscript{10,11} However, such a chromosomal band may contain 1,000 to 10,000 kb of information in addition to the Ig locus. Yet, examination of Burkitt’s lymphomas revealed that their chromosomal breakpoint at 14q32 was actually situated within the H chain gene locus, with the breakpoint frequently occurring between the J\textsubscript{H} and C\textsubscript{H} regions.\textsuperscript{36,37} In this study, we have shown that SU-DHL-6 has one normally rearranged H chain allele which accounts for its IgM production. The other allele has a J\textsubscript{H} region which unexpectedly falls on two separate BamHI restriction fragments. This observation has allowed us to clone the reciprocal breakpoints of this chromosomal translocation and should enable us to determine the identity of the information being introduced from chromosome 18.\textsuperscript{29} The presence of this definable breakpoint within a DHL cell line provides the opportunity to assess the contribution of this translocation to the malignant phenotype of diffuse as well as follicular lymphoma.\textsuperscript{38}

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